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Beauveria bassiana yeast phase on agar medium and its pathogenicity against *Diatraea saccharalis* (Lepidoptera: Crambidae) and *Tetranychus urticae* (Acari: Tetranychidae)

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Abstract

Beauveria bassiana colonizes insect hosts initially through a yeast phase, which is common in some artificial liquid cultures, but not reported on artificial solid media. We describe a yeast-like phase for *B. bassiana* isolate 447 (ATCC 20872) on MacConkey agar and its virulence toward *Diatraea saccharalis* and *Tetranychus urticae*. The yeast-like cells of *B. bassiana* developed by budding from germinating conidia after 24-h incubation. Cells were typically 5–10 μm and fungal colonies were initially circular and mucoid, but later were covered with mycelia and conidia. Ability to produce yeast-like cells on MacConkey medium was relatively common among different *B. bassiana* isolates, but growth rate and timing of yeast-like cell production also varied. *Metarhizium anisopliae* and *Paecilomyces* spp. isolates did not grow as yeast-like cells on MacConkey medium. Yeast-like cells of *B. bassiana* 447 were more virulent against *D. saccharalis* than conidia when 10^7 cells/ml were used. At 10^8 cells/ml, the estimated mean survival time was 5.4 days for the yeast suspension and 7.7 days for the conidial suspension, perhaps due to faster germination. The LC_{50} was also lower for yeast than conidial suspensions. Yeast-like cells and conidia had similar virulence against *T. urticae*; the average mortalities with yeast-like cells and conidia were, respectively, 42.8 and 45.0%, with 10^7 cells/ml, and 77.8 and 74.4%, with 10^8 cells/ml. The estimated mean survival times were 3.6 and 3.9 for yeast and conidial suspensions, respectively. The bioassay results demonstrate the yeast-like structures produced on MacConkey agar are effective as inoculum for *B. bassiana* applications against arthropod pests, and possibly superior to conidia against some species. Obtaining well-defined yeast phase cultures of entomopathogenic hyphomycetes may be an important step in studies of the biology and nutrition, pathogenesis, and the genetic manipulation of these fungi.

1. Introduction

The fungus *Beauveria bassiana* is widely distributed in all regions of the world and can be isolated from insects, mites, and soil, where this fungus is a part of the normal microbial flora, and other substrates (Boucias and Pendland, 1998). This species has a large genetic variation among the different isolates. Pathogenicity and virulence to different arthropods as well as enzymatic and DNA characteristics vary among different isolates (Almeida et al., 1997; Moino et al., 1998). Under certain climatic conditions, this fungus can cause natural epizootics in insect populations, as has been observed for *Anthonomus grandis*, *Cosmopolites sordidus* (Coleoptera: Curculionidae), *Hypothenemus hampei* (Coleoptera: Scolytidae), *Nezara viridula* (Hemiptera: Pentatomidae), *Diatraea saccharalis* (Lepidoptera: Crambidae), *Heterotermes tenuis* (Isoptera: Rhinotermitidae), *Castnia licus* (Lepidoptera: Castniidae), *Brassolis* spp. (Lepidoptera: Nymphalidae), and other important pests in Brazil (Alves, 1998).

The life cycle of *B. bassiana* in an arthropod host is initiated with germination of conidia that contact the host integument and produces a germ-tube that penetrates the host. The fungus colonizes the host initially through a yeast phase, which for most entomopathogenic fungi is an

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obligatory parasitic phase not normally observed outside the insect host (Alves, 1998; McCoy et al., 1985). Certain fungi will have this yeast phase when cultured in artificial liquid media, which mimic the insect hemolymph (Jackson, 1997). However, *Nomuraea rileyi* produces the yeast phase on artificial agar media (Pendland and Boucias, 1997). Although yeast-like forms with varying sizes, shapes, and nuclear characteristics have been reported for *B. bassiana* in insects and artificial liquid culture (Lima and Tigano, 1989), no references to the occurrence of the yeast phase on artificial solid media have been reported for the genus *Beauveria*.

Little is known about the morphology and the factors that regulate the development of entomopathogenic fungi (Pendland and Boucias, 1997), especially in relation to possible yeast-like phases of the hyphomycetes such as *B. bassiana*, *Metarhizium anisopliae*, and *Verticillium lecanii*. For *N. rileyi*, the yeast phase is common on solid media and the hyphal bodies develop into mucoid colonies that may last 1–7 days, depending on the isolate, before development of mycelia and conidia (Pendland and Boucias, 1997). The loss of virulence in *N. rileyi* isolates has been correlated with the absence of the yeast-like phase and may occur after serial passages in artificial media (Morrow et al., 1989).

Obtaining well-defined yeast-phase cultures of entomopathogenic hyphomycetes may be an important step in studies of the biology, nutrition, pathogenesis, and in genetic manipulation of these fungi. Understanding of the biology of the yeast phase may provide insights into a more efficient use of these entomopathogenic fungi, with implications in mass-production, use in microbial control programs, and in studies on virulence. Our objective was to study the occurrence of a yeast-like phase for *B. bassiana* isolate 447 and other entomopathogenic fungi on an agar medium. The virulence of this growth phase was tested against *D. saccharalis* and *Tetranychus urticae* because these insects are susceptible to this *B. bassiana* isolate, and are maintained in laboratory cultures at the Entomology Section at ESALQ-Univ. of São Paulo where experiments were conducted.

2. Materials and methods

2.1. Description of the cycle of yeast-like phase for B. bassiana isolate 447

Beauveria bassiana isolate 447 (ATCC 20872) is a standard commonly used in insect bioassays in our laboratory. This isolate, obtained from infected fire ant in Brazil, has been used in the microbial control of fire ants in the USA (Pereira et al., 1993). Isolate 447 is maintained as pure conidia at −12 °C in the Entomopathogen Collection maintained in the Insect Pathology and Microbial Control Laboratory, Department of Entomology, Phytopathology and Agricultural Zoology at the Escola Superior de Agricultura "Luiz de Queiroz," Universidade de São Paulo, (ESALQ-USP) Piracicaba, Brazil. The isolate 447 was cultured on potato–dextrose–agar (PDA) plates and conidia were produced by subculturing the fungus on a sporulation medium containing 0.36 g KH_2PO_4, 1.05 g $Na_2HPO_4 \cdot 7H_2O$, 0.6 g $MgSO_4 \cdot 7H_2O$, 1 g KCl, 10 g glucose, 1.58 g $NaNO_3$, 5 g yeast extract, and 20 g agar in 1000 ml water. Conidia were suspended in sterile water + Tween 40 (10^8 conidia/ml). Aliquots of 0.1 ml were inoculated on 10-cm plates containing MacConkey agar medium + crystal violet (Sanofi Diagnostics Pasteur) (17 g peptone, 3 g proteose peptone, 10 g lactose, 1.5 g bile salts, 5 g NaCl, 13.5 g agar, 0.03 g neutral red, and 1 mg crystal violet in 1000 ml water). The conidial suspension was spread on the agar surface using a glass rod and the plates were maintained in an incubator at 26 ± 1 °C and 12-h photophase.

Observations were made on fungal development, with both dissecting and optical microscope at 12, 32, 48, 60, 72, 84, 96, and 168 h after inoculation of the plates. Small fragments of the fungal colonies in lactophenol blue were prepared on microscope slides and covered with glass coverslip for examination. At 60 h after inoculation, the colonies were also examined using a scanning electronic microscope (SEM). A suspension containing yeast-like cells was prepared and spread on glass coverslips. The material was then fixed over osmium tetroxide vapor (OsO_4) for 24 h and then dried for 72 h in a glass desiccator with silica-gel (Quattlebaum and Garner, 1980), gold-coated in a sputter coater Balzers (model MED 010) for 120 s, and observed with SEM model LEO 435 VP.

2.2. Yeast-like growth among isolates of B. bassiana and other entomopathogenic fungi

Fifteen other isolates of *B. bassiana* (Table 1) were inoculated on MacConkey agar medium + crystal violet (BBL, Becton–Dickinson), maintained in incubator at 26 ± 1 °C, and observed daily under both dissecting and optical microscopes for the presence of yeast-like cells. Colonies grown on Sabouraud's dextrose agar medium (SDA) were used as standards for normal fungal growth. Four *B. bassiana* isolates were also inoculated onto SDA amended with 0, 0.25, 0.5, 1, 2, or 3% sodium chloride (SDA + NaCl) and observed as above. Because MacConkey medium contains 0.5% NaCl and 0.15% bile salts No. 3, we hypothesized that occurrence of a yeast-like phase was dependent on osmotic concentration, or other variable affected by the salt presence. Observations were also made on isolates of *M. anisopliae* (24 isolates) and *Paecilomyces* spp. (two isolates) (Table 1) grown on the MacConkey medium.

Table 1
Yeast-like growth of fungal isolates tested on MacConkey agar medium

Isolate number[a]	Source	Location	Collection date	Yeast-like growth[b]
B. bassiana				
16.97	Soil	Knoxville, TN	May 1997	*
200.97	Soil	Knoxville, TN	July 1997	–
267.97	Soil	Knoxville, TN	July 1997	–
1.98	Mycotrol ES	Commercial product, isolate GHA	December 1997	**
4.98	Diptera: Tipulidae	Wartburg, TN	June 1998	–
5.98	Aquatic insect larva	Wartburg, TN	June 1998	–
9.98	[c]	[c]	[c]	–
11.98	Coleoptera: Elateridae	Huntsville, TN	June 1998	***
12.98	*Camponotus* sp.	Bristol, TN	June 1998	**
13.98	*Leptinotarsa decemlineata*	Knoxville, TN	June 1998	**
15.98	Hymenoptera: Sphecidae		August 1998	***
4.99	*Macrosiphum euphorbiae*	Crossville, TN	January 1999	**
1.00	*Popillia japonica*	McMinnville	2000	–
ARSEF 201	*Diabrotica undecimpunctata*	Corvalis, OR	October 1977	–
ARSEF 2270	*Cyrtepistomus castaneus*	Breathitt, KY	September 1986	–
M. anisopliae				
261.97	Soil	Knoxville, TN	July 1997	–
312.97	Soil	Knoxville, TN	July 1997	–
14.98	*Leptinotarsa decemlineata*	Knoxville, TN	June 1998	–
2.99	*Solenopsis* sp. reproductive female	Loudon, TN	February 1999	
798	*Solenopsis invicta*	Cuiabá, MT, Brazil	July 1988	–
816	Soil	Piracicaba, SP, Brazil	August 1988	–
860	*Macropsis* sp.	Piracicaba, SP, Brazil	February 1989	–
865	Hemiptera	Goiânia, GO, Brazil	March 1989	–
1022	*Phyllophaga* sp.	Piracicaba, SP, Brazil	December 1991	–
1027	Soil	Boa Esperança, PR, Brazil	December 1991	–
1037	*Solenopsis* sp.	Porto Alegre, RS, Brazil	March 1992	–
1104	Soil	São João do Piauí, PI, Brazil	August 1992	–
1144	*Calosoma granulata*	Piracicaba, SP, Brazil	April 1994	–
1172	Soil	Córrego Rico, SP, Brazil	November 1994	–
1175	Soil	Córrego Rico, SP, Brazil	November 1994	–
1189	Soil	Corumbá, MS, Brazil	June 1996	–
1203	Blattodea	PE, Brazil	August 1996	–
1204	*Mahanarva fimbriolata*	Piracicaba, SP, Brazil	October 1996	–
1247	Soil	Turvínia, SP, Brazil	April 1999	–
E6	*Diatraea saccharalis*	PE, Brazil	April 1981	–
E9	*Mahanarva posticata*	Boca da Mata, AL, Brazil	May 1981	–
PL43	*Mahanarva* sp.	Fleixeira del Estado, AL, Brazil	August 1981	
RJC	Laboratory mutant	Piracicaba, SP		–
RJD	Laboratory mutant	Piracicaba, SP		–
Paecilomyces spp.				
7.97	Soil	Knoxville, TN	July 1997	–
175.97	Soil	Knoxville, TN	July 1997	–

[a] Isolates #.97 to #.00 are from the fungal isolate collection maintained at the Department of Entomology and Plant Pathology, University of Tennessee; ARSEF isolates are from the Collection of Entomopathogenic Fungal Cultures maintained by the USDA Plant, Soil, and Nutrition Laboratory in Ithaca, NY; other isolates are from the Entomopathogen Collection maintained in the Insect Pathology and Microbial Control Laboratory, Department of Entomology, Phytopathology, and Agricultural Zoology at the Escola Superior de Agricultura "Luiz de Queiroz," Universidade de São Paulo, (ESALQ-USP) Piracicaba, Brazil.

[b] ***, Abundant yeast-like growth up to 6 days after plate inoculation; **, initial yeast-like growth until 4 days after plate inoculation; *, limited yeast-like growth 4 days after plate inoculation; –, no yeast-like growth observed.

[c] Records have been lost for this isolate but it was derived from either isolate ARSEF 3216 isolated in Rusk, WI, from *Thrips calcaratus* (Summer 1991), or isolate ARS 3385 isolated in Yakima, WA, from *Myzus persicae* (July 1991).

2.3. Bioassays with D. saccharalis

Yeast-like cells of *B. bassiana* isolate 447 obtained from 65-h cultures on MacConkey agar plates were used in bioassays against laboratory-reared, third-instar *D. saccharalis* (King and Hartley, 1985). Preliminary experiments were conducted by immersing 10 larvae for 3 s in a suspension containing 10^8 yeast cells/ml. After inoculation, the larvae were placed individually in plastic petri dishes (10-cm diameter, 2-cm height). Larvae were

offered corn stalk segments for the first 4 days after inoculation and then sugarcane segments for the duration of the experiments. Test larvae were maintained in incubators, as described above.

After demonstration of yeast-like cell pathogenicity, dose–response bioassays were conducted. Suspensions were prepared containing either yeast-like cells or conidia obtained from MacConkey agar. Conidia were obtained after 180-h incubation. Concentrations of 10^5, 10^6, 10^7, and 10^8 cells/ml were used, for a total of eight treatments plus one control with sterile water + Tween 40. Each treatment consisted of three replicates of 10 third-instar *D. saccharalis* sprayed together with 3 ml fungal suspensions using a Potter Tower (Burkard Manufacturing Company) at 15 lb/in.^{2}.

After treatment, the larvae were placed individually in plastic petri dishes maintained, and fed, as described previously. Daily observations and removal of cadavers continued for 10 days with a final observation on day 15. Insect cadavers were washed for 30 s in 70% ethanol followed by 1 min in distilled water and placed in a humid chamber to allow growth of infecting fungus. Insects showing fungal growth after incubation at 26 °C and 12-h photophase for 7–10 days were recorded as killed by the fungus. Cumulative percent mortalities after 10 and 15 days were corrected using Abbott's formula (1925). Data were analyzed after arcsin transformation and treatment means were compared using Fisher's protected least significant differences. The mean survival time (MST) was calculated based on the number of days after treatment when individual insects died. MST for insects treated with 10^8 yeast-like cell or conidial suspensions was compared using a one-tailed *t*-test. Cumulative mortalities were used to estimate the concentration (LC_{50}) necessary to kill 50% of the treated population, using Probit analysis (Polo PC Software).

2.4. Bioassays with T. urticae

Bioassays were conducted using newly eclosed *T. urticae* adult females reared in the laboratory on jack bean plants, *Canavalia ensiformis* (L.) DC. (Dicotyledonea: Fabaceae). Fifteen mites were transferred to 4-cm plastic petri dishes containing a moist polyethylene sponge disk. Each disk with mites was sprayed with 2 ml treatment suspensions using a Potter Tower at 15 lb/in.^{2}. The suspensions used in the treatments and control were those described above. Two hours after inoculation, the number of mites per disk was reduced to 10 by removal of dead mites or any extra living mite. Ten disks were used per treatment, for a total of 100 mites per treatment. The petri dishes were placed in transparent plastic boxes (34 cm × 22 cm × 12 cm) closed with lids and placed in incubator at 26 ± 1 °C, 98% RH, and 12-h photophase. Daily observations, removal, and handling of cadavers were as described previously but only for 5 days. The experiment was repeated using identical methods. Mite mortality results for corresponding doses were similar in both experiments so data were combined before statistical analysis. Cumulative percent mortalities after 5 days were corrected (Abbott, 1925) and all data were analyzed as described above, except that MST was calculated using the average survival time for mites placed in each leaf.

3. Results and discussion

3.1. Description of the yeast phase of B. bassiana isolate 447 on MacConkey agar

The yeast-like cells of *B. bassiana* that developed from germinating conidia (unipolar, bipolar, and tripolar) were observed after 24-h incubation on MacConkey agar. Yeast cells developed by budding from the germ-tubes after 32 h. At this time, the fungal colonies could not be observed without a microscope. These yeast-like cells were typically 5–10 μm long (Fig. 1A). After 32–72 h, long narrow structures similar to hyphae were formed, but these produced either yeast-like cells or other structures that resembled blastospores. During this phase, fungal colonies that spread over the agar plate surface could be observed macroscopically (Fig. 1B). Colonies were circular and varied in size (typically 1–3 mm diameter), with a mucoid appearance similar to that of young *N. rileyi* colonies (Pendland and Boucias, 1997).

After 80–96 h, aerial mycelium started to form over the colonies on the agar plates. The fungal colonies lost the mucoid appearance after 120 h of incubation when the mycelia covered most of the agar plate (Fig. 1C). After 168 h, conidiogenesis started (Fig. 1D) and typical *B. bassiana* zigzag conidiogenous cells could be observed. The growth cycle for isolate 447 on MacConkey medium (Fig. 2) was similar to that of other *B. bassiana* isolates included in this study that produced yeast-like cells.

Similar yeast-like structures have been observed in several liquid media (Lima and Tigano, 1989). These structures occurred with varying frequencies in different media and were elongated, with varying sizes, thin cell wall, uninucleate, but becoming multinucleate due to the occurrence of repeated mitoses. Similar structures were also observed inside the insect host *Spodoptera frugiperda* (Lepidoptera: Noctuidae) 36 h after inoculation. The structures in the insect were described as cylindrical, with 1–7 nuclei. The yeast-like forms are important in studying genetic variability and in obtaining protoplasts, which are essential for fungal parasexuality and genetic transformation studies (Lima and Tigano, 1989). Studies are under way to determine the cell wall composition of the *B. bassiana* yeast-like structures produced on

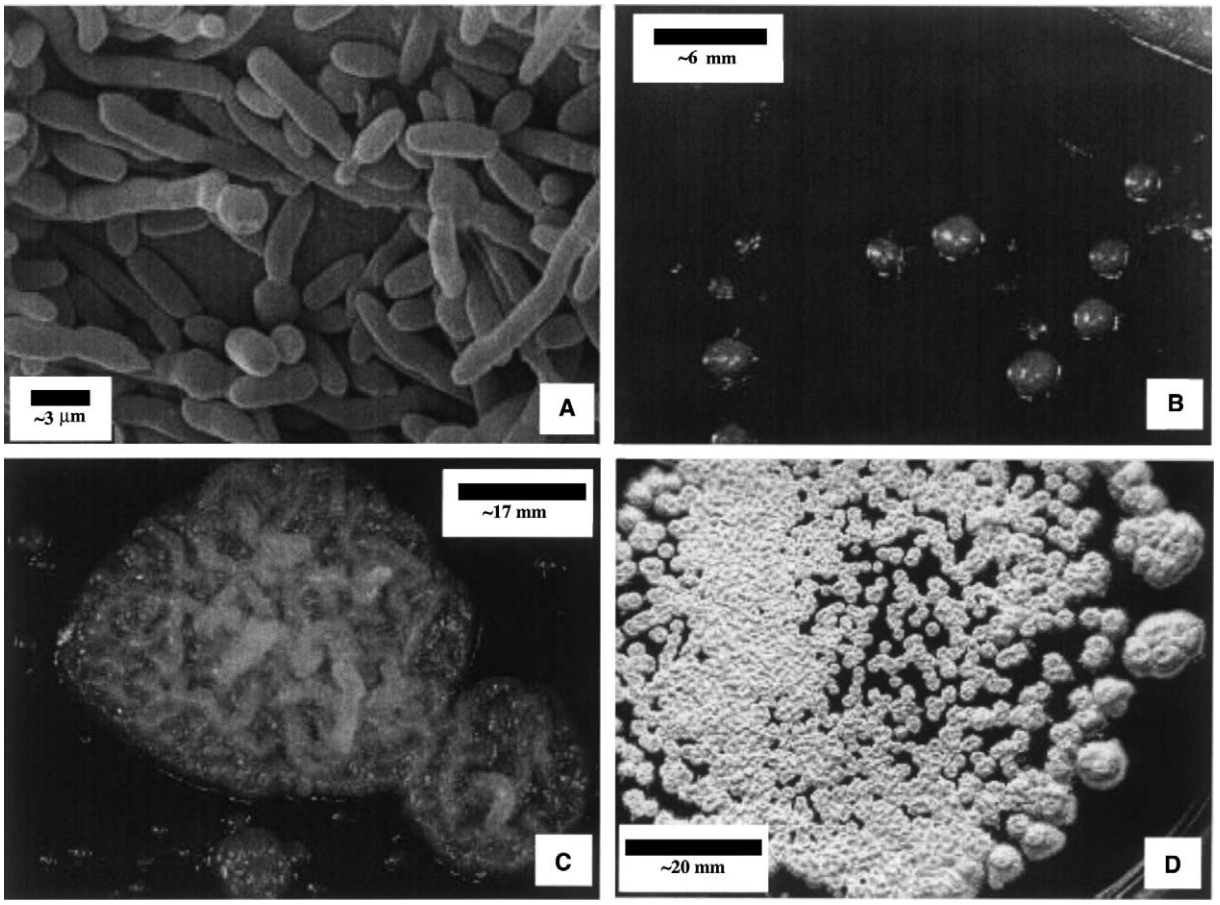

Fig. 1. (A) Yeast-like cells of *B. bassiana* isolate 447 (SEM). Colonies of *B. bassiana* isolate 447 on MacConkey agar medium; (B) 3-day-old colonies; (C) 5-day-old colonies with mycelial growth; (D) 10-day-old colonies covered with conidia.

MacConkey agar. Cell wall composition is important in eliciting insect immune system reaction and may determine the fate of pathogen in different hosts.

3.2. Yeast-like growth among isolates of B. bassiana and other entomopathogenic fungi

Six of the 15 *B. bassiana* isolates tested grew as yeast-like colonies on MacConkey medium (Table 1). Colonies of isolates 11.98 and 15.98 were predominantly yeast-like up to 6 days after inoculation. Other isolates, which grew initially as yeast-like colonies (1.98, 12.98, 13.98, and 4.99), switched to mycelial growth by day 4 and mycelia predominated on the colonies by day 6. Isolate 16.97 had very slow growth on MacConkey medium but yeast-like growth was observed by day four. No yeast-like colonies were observed on eight isolates (200.97, 267.97, 4.98, 5.98, 9.98, 1.00, ARSEF 201, and ARSEF 2270), although a few yeast-like cells could be observed near the mycelial tips 3 and 4 days after inoculation of plates. None of the *M. anisopliae* or *Paecilomyces* spp. isolates grew yeast-like cells on MacConkey medium.

Isolate 5.98 grew few yeast-like cells on SDA containing NaCl (0.25–3%) restricted to area along mycelial branches. Colonies predominantly with yeast-like cells were never observed for this isolate. Other isolates grown on SDA + NaCl, including 11.98 and 15.98 which grew as yeast-like colonies on MacConkey medium, and 16.97 that showed a late initiation of yeast-like growth, did not produce yeast-like growth on SDA + NaCl. The addition of salt to SDA caused appearance of yeast-like growth, even with isolate that did not show yeast-like cells on MacConkey medium (5.98). Yeast-like growth may be related to salt concentration, and perhaps to osmotic pressure, in the medium. However, the different salt concentrations used did not correspond to different levels of yeast-like growth. We will examine the key factors determining the appearance of yeast-like growth of *B. bassiana* on agar media in future experiments.

Results with different isolates of *B. bassiana* demonstrate that the ability to produce yeast-like cells on MacConkey medium is relatively common among different isolates. However, the growth rate and timing of yeast-like cell production also varied among the isolates. It is not known whether the ability of a *B. bassiana*

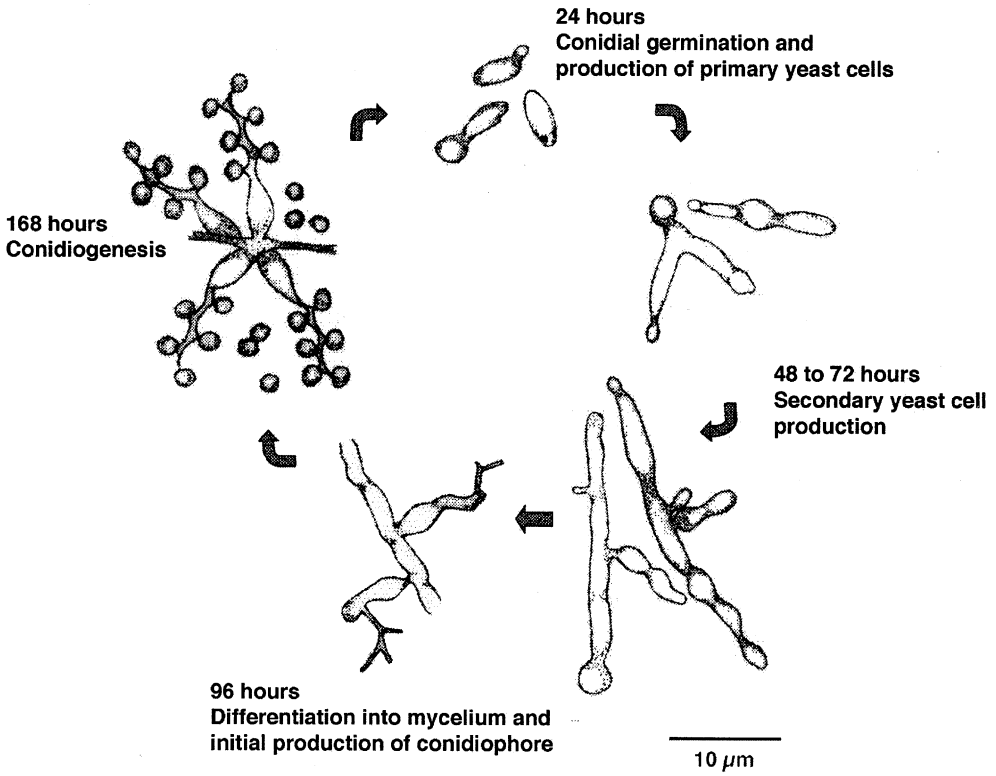

Fig. 2. Growth cycle of *B. bassiana* isolate 447 on MacConkey agar medium.

isolate to grow as yeast-like cells on agar medium is related to its virulence, as observed for *N. rileyi* (Morrow et al., 1989). *M. anisopliae* and *Paecilomyces* spp. isolates tested did not seem to grow as yeast-like cells on MacConkey medium. Although 24 *M. anisopliae* isolates were tested, conditions necessary to induce yeast-like growth on this fungus may be outside the range we tested. Also, other isolates of *Paecilomyces* sp. may behave differently from the two isolates tested.

3.3. Pathogenicity of B. bassiana yeast-like cells and conidia to D. saccharalis

With all the treatments used in bioassays, mortality increased with time and with doses of *B. bassiana* (Fig. 3), regardless of the structure (yeast-like cells or conidia) used to inoculate insects. Mortalities in treatments with 10^7 and 10^8 cells/ml were significantly higher than the control mortality after 15 days. At low concentration (10^5 or 10^6 cells/ml), relatively few *D. saccharalis* larvae showed any signs of fungal infection after incubation of cadavers, despite Abbott-corrected mortalities between 2 and 25%. Because a small inoculum was used, other microbes may have taken over the insect cadavers before sufficient development of the fungus.

With the high concentrations (10^7 and 10^8 cells/ml), the suspensions containing yeast-like cells caused higher mortality than the suspensions containing conidia. With

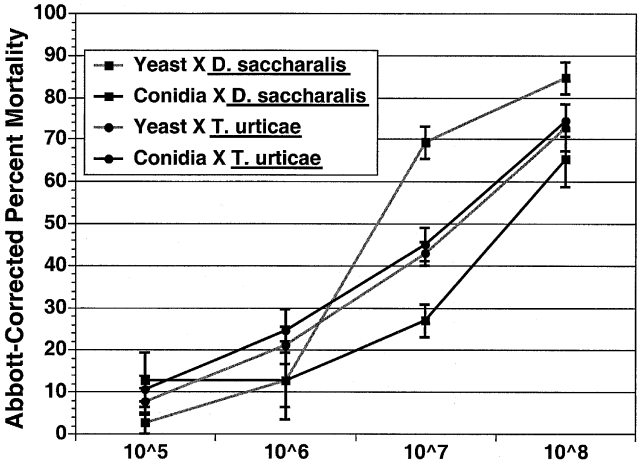

Fig. 3. Mean Abbott-corrected mortality 15 days after treatment of third-instar *D. saccharalis* and adult *T. urticae* with *B. bassiana* (isolate 447) yeast-like cells or conidia produced on solid agar medium. Error bars represent standard error of the mean.

the 10^7 suspension, yeast-like cell suspensions killed approximately 70% of the treated insects compared to less than 30% killed by the conidial suspension with equivalent concentration. Also with the 10^7 suspension, the number of cadavers showing signs of *B. bassiana* growth was 40% higher with yeast-like cell suspension than with conidial suspension. The time required to produce mortality was also shorter than that for

conidial suspension, demonstrating the effectiveness of the yeast-like cell inoculum. Hyphal bodies of *Paecilomyces fumosoroseus* (Fargues et al., 1994) also caused higher mortality than ungerminated conidia when applied against *S. frugiperda.*

With the 10^8 cells/ml suspension, there was no significant difference in mortality between the inoculum types (Fig. 3). However, the MST was significantly shorter ($t = 2.309$, $P = 0.0129$, $df = 43$) for the yeast suspension (5.4 days) than for the conidial suspension (7.7 days) (Table 2). Shorter MST may be due, at least partially, to a shorter time required for full germination, as demonstrated for *B. bassiana* blastospores produced submerged in liquid medium (Thomas et al., 1987) and for *P. fumosoroseus* (Jackson, 1997; Jackson et al., 1997). These results demonstrate a higher virulence of the yeast-like structures produced on MacConkey agar plates when compared to conidia produced under the same conditions. The estimated LC_{50} was lower for applications of conidia than for yeast suspensions. *B. bassiana* aerial conidia have been shown to be more efficient than blastospores against leafhoppers due to better adhesion to the host provided by the hydrophobic nature of the conidia (Lane et al., 1991). However, for some insects, such as sessile whitefly nymphs, spore adhesion may not be as important as with mobile insects (Jackson et al., 1997). The use of a caterpillar host, which has little ability to groom itself compared to other insects, may have provided favorable conditions for fungal development.

3.4. Pathogenicity of *B. bassiana* yeast-like cells and conidia to *T. urticae*

The *T. urticae* mortalities caused by yeast and conidial suspensions also increased with an increase of the concentration, as observed with *D. saccharalis* (Fig. 3). However, mortalities caused by yeast and conidia suspensions were very similar for all the concentrations. With concentrations of 10^7 cells/ml, the average Abbott-corrected mortality was 42.8% for the yeast suspension and 45.0% for the conidial suspension. With concentrations of 10^8 cells/ml, the averages were 77.8% for the yeast suspension and 74.4% for the conidial suspension.

With the 10^8 cells/ml suspension, the estimated MSTs for the yeast and conidial suspensions were considerably shorter than those observed against *D. saccharalis* (Table 2). Results for LC_{50} were not different for the two inoculum types, but MSTs were significantly different ($t = 2.557$, $P = 0.0075$, $df = 36$). The yeast inoculum had slightly shorter MST (3.6 days) and smaller LC_{50} (12.4×10^6 yeast-like cells/ml) than those for conidial suspension (MST = 3.9 days; $LC_{50} = 12.6 \times 10^6$ conidia/ml).

Despite a slightly shorter MST, the results for *T. urticae* bioassays did not demonstrate a higher virulence of the yeast-like structures as observed with *D. saccharalis*, but indicate that the yeast-like cells can be effective inoculum for *B. bassiana* applications against very different arthropods. Differences in the host cuticle characteristics and the hydrophobicity of yeast-like cells and conidia may play important role in the adhesion of these infective units to the host. Higher activity of yeast-like cells against *D. saccharalis* may be due to better adhesion and penetration of these cells on the caterpillar cuticle. Adhesion and growth of yeast-like cells on insect cuticle may have been enhanced by the use of aqueous suspensions in spraying the test insects. Aqueous suspensions, although normally used in entomopathogenic fungal applications, may have detrimental effect on adhesion of the hydrophobic conidia.

The advantages of yeast-like cell applications need to be further explored under different systems, including studies on mass-production (Jackson, 1997). The shorter time required for production of yeast-like cells, compared to production of conidia, may significantly lower production costs of entomopathogenic fungi used for microbial control. As demonstrated in the experiments reported here, it is possible that the yeast phase will be more efficient against some insects than the aerially produced conidia. Comparisons of virulence should also be pursued between conidia and blastospores produced submersed in liquid culture (Thomas et al., 1987) and the yeast-like cells reported here. If reliable systems can be

Table 2
Mean survival time (MST), LC_{50}, and relative potency of *B. bassiana* (isolate 447) yeast-like cells or conidia produced on solid agar medium applied against third-instar *D. saccharalis* and newly eclosed female *T. urticae*

	Yeast		Conidia		Relative potency (yeast/conidia)	
	MST (days)[a]	LC_{50}[b] ($\times 10^6$ cells/ml)	MST_{50} (days)	LC_{50} ($\times 10^6$ cells/ml)	LT_{50}	LC_{50}
D. saccharalis	5.42 ± 0.65	5.6[c]	7.67 ± 0.72	4.8[c]	1.81	0.86
T. urticae	3.56 ± 0.09	12.4 (8.0–20.1)	3.91 ± 0.11	12.6 (7.8–22.2)	1.11	1.02

[a] Mean survival time (mean ± SEM) estimated for the insects treated with suspensions containing 10^8 cells/ml.

[b] Lethal concentration for 50% of the treated population [mean (fiducial limits)] based on cumulative mortalities after 15 and 5 days, respectively, for *D. saccharalis* and *T. urticae*. For *T. urticae*, the slopes were 0.77 and 0.66, and χ^2 values were 1.11 and 0.43 for yeast-like cells and conidia, respectively.

[c] Large data variation did not allow estimation of confidence interval.

designed for mass-production and formulation (Fargues et al., 1983) of this yeast inoculum, its use in microbial control programs can be advantageous in comparison with conidia, at least against some pest species. Methods that guarantee the survival of yeast-like cells in the environment would be necessary if these propagules are to be used in microbial control programs.

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